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GENETIC DIVERGENCE AND LOSS OF DIVERSITY IN TWO CULTURED POPULATIONS OF THE BAY SCALLOP, *ARGOPECTEN IRRADIANS* (LAMARCK, 1819)

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ABSTRACT Researchers at the Virginia Institute of Marine Science (VIMS) have been maintaining a small-scale bay scallop (*Argopecten irradians*) culturing operation since the late 1960s. The cultured line was originally established with broodstock collected from the coasts of Virginia and North Carolina, but it has since been augmented with a ''grab bag'' of introductions from other source populations. A large bay scallop–culturing operation was reportedly founded in China in the early 1980s, with 26 individuals provided by the VIMS researchers. The degree of genetic divergence between these two populations since the founding of the Chinese operation is unknown, as are the relative amounts of genetic diversity that may have been maintained under the selective pressures of the hatchery.

Samples of cultured bay scallops were obtained from culturing operations in Wachapreague, VA, in 1993 and 1995, and from the Shandong Province of China in 1993. Mitochondrial DNA (mtDNA) was isolated from individual scallops, digested with a battery of eight restriction enzymes, and analyzed by restriction fragment length polymorphism analysis. Measures of haplotype diversity and divergence were calculated for the samples to reveal genetic differences between the cultured populations and to allow comparison of the levels of genetic variation maintained in the cultured populations relative to those observed in several natural populations of bay scallops. A sample of 55 Virginia cultured bay scallops was found to be monotypic, represented by a single haplotype, and three haplotypes were observed in 36 individuals sampled from China. No haplotypes were shared between the samples, indicating that significant divergence has occurred between the populations. The single haplotype from Virginia was observed in a sample of bay scallops from New England, and the least common haplotype from the Chinese sample was also found in samples from New England, North Carolina, and Crystal River, FL. Haplotype diversity and genotypic divergence values for the cultured samples indicate that mtDNA variation may be lost in the culturing process and that a bottleneck effect and/or genetic drift has affected the levels of variation in these populations differently. Assuming that the Chinese culturing operation was founded exclusively with individuals from the Virginia population, it can be concluded that the latter has lost a greater proportion of the original variation in the intervening generations of hatchery breeding.

KEY WORDS: Bay scallop, aquaculture, genetics, mtDNA variation, inbreeding

INTRODUCTION

The bay scallop, *Argopecten irradians* (Lamarck), is endemic to shallow estuarine habitats along the East Coast of the United States, from Massachusetts to Texas (Clarke 1965). Four subspecies have been described based on shell morphometrics (Waller 1969, Petuch 1987), although both restriction fragment length polymorphism (RFLP) analysis of mitochondrial (mt) DNA (Blake and Graves 1995) and allozyme studies (Marelli et al. 1997) have indicated that individuals described as the subspecies *A. i. taylorae* are genetically indistinguishable from *A. i. concentricus*. The bay scallop has been fished commercially since the mid-1800s, and it also supports a large recreational fishery (Shumway and Castagna 1994). Because populations appear to be recruitment limited (Peterson and Summerson 1992) and highly variable in size, the potential for aquaculture of the species has received considerable attention.

The first significant attempt to rear cultured bay scallops to market size was undertaken by Castagna and Duggan (1971) in the late 1960s. The initial stock for the study consisted of 66 adult bay scallops (*A. i. concentricus*) collected from bays along the Eastern Shore of Virginia and from Bogue Sound, NC. In the years since the establishment of this original cultured line, the broodstock has been supplemented with individuals from Massachusetts (*A. i. irradians*) and Texas (*A. i. amplicostatus*) (M. Castagna, Virginia Institute of Marine Science [VIMS] 1993, pers. comm.). These additions served to contaminate the line so that the exact subspecific composition of the current broodstock is unknown. This bay scallop–culturing effort has continued to the present day, coordinated by researchers from VIMS. A typical spawning protocol involves a mass, induced spawning of 100–200 broodstock animals, to produce an estimated 50–150 million eggs. Several such spawns may be performed and the resultant eggs pooled. A small commercial market has developed for the Virginia cultured product.

In 1982, 128 scallops from the VIMS Eastern Shore culturing operation were transported to laboratories in Qingdao, China, with the intent of establishing a bay scallop–culturing effort in the waters of China's eastern bays. Twenty-six of the transported individuals survived the journey to spawn in January 1983 (Chew 1990). By 1989, Chinese production of the ''Virginia'' American bay scallop exceeded 50,000 metric tons in-shell live weight (Chew 1990).

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The potential for loss of genetic variability due to inbreeding seems great for both the Virginia cultured line and that maintained in China. The relative degree and possible consequences of this loss are unknown. *A. irradians* is a functional hermaphrodite, and many of the larvae produced in the hatchery may be the result of facultative selfing. Inbreeding depression has been observed in self-fertilized larvae of the catarina scallop, *Argopecten circularis,* manifest as decreased larval growth and lower rates of survival (Ibarra 1995). Such inbreeding effects are thought to be a general danger for cultured species with very high fecundities, in which few individuals may produce large numbers of offspring (Newkirk 1978). The effective population size (N_e), or the number of broodstock individuals contributing gametes to the subsequent generation, may in fact be much smaller than the census number (N) of individuals used as broodstock in a hatchery (Gaffney et al. 1992). Culturing techniques in which parental individuals are mass spawned may exacerbate inbreeding problems, even when the number of resultant progeny is satisfactory.

By 1993, the Virginia and Chinese broodstocks had been isolated for 10 y and at least 10 generations, a period that should have permitted effects of the founding event and genetic drift to become apparent. It has been shown that RFLP analysis of the mtDNA reveals considerable genetic variation in natural populations of the bay scallop and that geographically isolated populations are genetically distinct (Blake and Graves 1995). In this study, a comparison of mtDNA variation in the Chinese and Virginia cultured populations, and in samples from bay scallop populations in their natural range, was undertaken to determine the genetic divergence between the Chinese and Virginia populations and the level of genetic variation maintained in the cultured populations relative to that in natural populations.

MATERIALS AND METHODS

A sample of 27 cultured bay scallops was provided by the VIMS laboratory at Wachapreague in March 1993. These were year-old individuals, progeny of broodstock spawned in April 1992. An additional sample of 28 individuals was obtained from the facility in March 1995. These were products of the April 1994 spawn and permitted comparison of temporally isolated samples of the Virginia cultured scallops.

Fresh tissue from 36 cultured bay scallops—18 from a northern growout site (Laizhou) and 18 from a growout site in Qingdao (Tiaonan)—was obtained from Chinese culturing facilities in October 1993. Although these individuals had been reared at the different sites, the seed originated from the same broodstock (X. Qinzhao, Institute of Oceanology, Academia Sinica, 1993, pers. comm.). Dissection of tissues from Chinese bay scallops was performed by the investigator (SGB), and confirmation that all were *A. irradians* was made at this time. The indigenous Chinese scallop, *Chlamys farreri,* is easily distinguished from the bay scallop, and none was included among the sampled individuals.

MtDNA was purified from scallop gonad, mantle, and gill tissue by cesium chloride density-gradient ultracentrifugation, as described in Blake and Graves (1995). The difficulties of transporting usable tissue from China to the United States made it necessary for initial preparative steps to be taken in the laboratories of the Institute of Oceanology in Qingdao, China. DNA isolation was initiated in China in early October 1993, but because equipment for ultracentrifugation was not available at this facility, the samples were maintained on ice (or orange-flavored ice pops, when ice was unavailable) after the addition of CsCl-saturated water to the tissue preparations (see Blake and Graves 1995). The samples were then transported to VIMS, where mtDNA was purified by cesium chloride density-gradient ultracentrifugation.

Purified mtDNA was digested with a battery of eight restriction enzymes for all individuals: *Ava*I, *Ban*I, *Ban*II, *Bgl*II, *Bst*EII, *Eco*RI, *Hae*II, and *Hin*dII. Restriction fragments were end-labeled with the Klenow fragment of DNA polymerase I and ^{35}S-labeled nucleotides, electrophoresed at 1 V/cm in 1% agarose gels overnight, and visualized by autoradiography (Sambrook et al. 1989). ^{35}S-labeled 1-kilobase ladder DNA (BRL) provided a molecular-weight size standard.

Sizes of mtDNA fragments were estimated by fitting band migration distances to those of the standard by the local reciprocal method of Elder and Southern (1983) by use of the program Gel Frag Sizer (Gilbert 1989). Restriction sites were inferred from completely additive fragment patterns, and letter designations were assigned to the different patterns. Eight-letter composite haplotypes were compiled for the series of enzymes and analyzed for site changes, following Blake and Graves (1995).

Statistical analyses were performed with the Restriction Enzyme Analysis Package (REAP) (McElroy et al. 1991). For each sample, haplotype and nucleotide diversities were calculated following the methods of Nei (1987) and Nei and Miller (1990), respectively. Mean nucleotide sequence divergence between samples was calculated following Nei and Miller (1990) and was corrected for within-population polymorphism by subtracting the average of within-sample diversities. Because several of the haplotypes observed were rare, a Monte Carlo simulation (Roff and Bentzen 1989) was performed to estimate heterogeneity and assess the likelihood that the sampled populations shared a common gene pool. Data from natural bay scallop populations (Blake and Graves 1995) were also used in comparative analyses with the Chinese and Virginia cultured bay scallop samples, to assess changes in diversity and divergence under culturing conditions.

RESULTS

DNA from a total of 91 cultured bay scallops was analyzed with eight restriction endonucleases, revealing four distinct mtDNA haplotypes (Table 1). The 1993 and 1995 samples of cultured bay scallops from Virginia were both monotypic, characterized by a single haplotype, AABAAAAE, and the two were combined into a single pooled sample (VA) of 55 monotypic individuals for further analysis. The haplotype diversity and mean nucleotide sequence diversity for the Virginia population were both calculated to be zero. The combined cultured Chinese sample (Q) comprised three haplotypes, none of which was identical to that observed in the Virginia sample.

A Monte Carlo test for heterogeneity was performed (Roff and Bentzen 1989) on the two subpopulations of bay scallops from China (Laizhou and Tiaonan), to determine whether these shared a common gene pool (originated from a common broodstock) and could be treated in subsequent analyses as one population. One thousand Monte Carlo randomizations yielded 126 χ^2 values exceeding the value from the original data, indicating that at $p = 0.126$, the populations are not significantly heterogeneous. The Chinese sample is hereafter discussed as a single population.

For the Chinese sample, haplotype diversity was 0.55 and mean nucleotide sequence diversity was 0.33%. The two less common haplotypes in the Chinese sample, ACCAAAAA and

TABLE 1.

A. irradians: composite haplotypes from two populations of cultured bay scallops and numbers of these haplotypes observed in five samples representing natural bay scallop populations.

		Cultured		"Natural"			
Haplotype	**Δ**	**Q**	**VA**	**MA**	**NC**	**FL**	**RK**
AABAAAAE	0		55	5			
AACAAAAA	2	4 (4)		7	26	2	
AABAAAEE	1	22 (9)					
ACCAAAAA	3	10 (5)					
Total n		36	55	26	48	27	34

Q, Qingdao, China; VA, Wachapreague, VA; MA, New England; NC, Harker's Island, NC; FL, Crystal River, FL; RK, Rabbit Key, FL. Restriction enzymes used: *Aval, Ban*I, *Ban*II, *Bgl*II, *Bst*EII, *Eco*RI, *Hae*II, and *Hind*II. Values in parentheses are totals from the Laizhou, China, growout facility. Δ is the number of site changes between the haplotype and the arbitrary standard of the single Virginia haplotype. A complete list of the haplotypes from the "natural" samples is provided in Blake and Graves (1995).

AACAAAAA, differed from each other by a single site change, whereas the third and most common, AABAAAEE, differed from these by several site changes. The latter, however, differed from the Virginia haplotype (AABAAAAE) by only one site change. The corrected mean nucleotide sequence divergence between the Virginia and Chinese samples was 0.13%.

Because there were no shared haplotypes between the Virginia and Chinese samples, it was not necessary to apply a rigorous test for heterogeneity to these two populations. Data from other bay scallop populations (Blake and Graves 1995) were used for comparison with the cultured samples of this study. Included in analyses were samples of natural populations from Harker's Island, NC, and Rabbit Key, FL. Hatchery-reared scallops from Woods Hole, MA (New England), and Crystal River, FL (Florida Gulf), were used to approximate genotype distributions for their regions of origin. These were not used in comparisons of genetic diversity. An abbreviated list of the genotypes found in the "natural" populations, including those found also in one of the cultured samples, is presented in Table 1.

Tests for heterogeneity were performed between the cultured samples and the sample from New England (Blake and Graves 1995), with which each shared a single haplotype. In both tests (MA and VA, and MA and Q), the 1,000 randomizations produced no χ^2 values higher than the observed, indicating that significant heterogeneity exists between the tested pairs. The least common haplotype from the Chinese sample (AACAAAA), represented by four individuals, was also present in the New England, North Carolina, and Crystal River samples. No other haplotypes were shared between the cultured samples and those representing natural bay scallop populations. Two of the three Chinese haplotypes were unique to that sample.

Corrected mean nucleotide sequence divergences between the cultured samples evaluated in this study and the natural populations previously described (Blake and Graves 1995) ranged between 0.04% (Q vs. MA) and 0.24% (Q vs. FL) (Table 2). Although still not sharing a common gene pool, the Chinese sample was found to be less divergent from the New England sample (0.04%), with which it shared a single common haplotype, than from the cultured Virginia population (0.13%).

DISCUSSION

The genetic aspects of hatchery rearing of bay scallops are of great interest to culturists in the United States, where the bay scallop is native, and China, where culture of this scallop is being undertaken on a very large scale. A loss of genetic variation due to drift is apparent in both the Chinese and the Virginia cultured lines, although most notably so in the latter, which has apparently become fixed for a single mtDNA haplotype. The Chinese population represented by the Qingdao sample also possessed a lower haplotype diversity (0.55) than natural bay scallop populations from Rabbit Key (0.91) and North Carolina (0.69, pooled) (Blake and Graves 1995). The strategy of broadcast spawning appears to be very effective at maintaining genetic diversity for the bay scallop in nature.

It can be stated with confidence that at the founding of the Virginia line, there was a greater level of genetic diversity present in the broodstock than was measured in this study. MtDNA analyses of a natural population from North Carolina, one of the putative sources of the Virginia line, revealed considerably higher haplotype diversities (Blake and Graves 1995). In two samples from North Carolina, the haplotype diversity, or probability of encountering different haplotypes when two individuals are sampled from a population, ranged between 0.63 and 0.74. Even if hatchery rearing has reduced this level in the Virginia cultured line, periodic introductions from other source populations should have served to reintroduce genetic variability to the population.

The samples obtained from the Virginia culturing facility originated from mass spawnings of 100–200 animals. The numbers of contributing parent individuals are not precisely known, but it is apparent from the current monotypic state of the population that differential reproductive success has occurred during one or more of these spawning events. A single cataclysmic loss may have occurred in which one or very few maternal individuals contributed to the subsequent generation. Similarly, a series of less dramatic losses may have occurred, to bring the population to fixation over a period of generations.

The initial bottleneck of no more than 26 breeding individuals that established the Chinese culturing operation in 1983 was not sustained, because the production of bay scallops in China grew extremely rapidly. If the 26 transplanted individuals reflected all of the genetic variation present in the Virginia source population at the time, it would appear that Chinese culturing methods have been more conducive to a maintenance of that variation. This tendency for loss under the Virginia hatchery regimen may be even more pronounced, if subsequent additions have been made to the Vir-

TABLE 2.

A. irradians: matrix of nucleotide sequence divergences among populations, in percents, corrected for within-sample variation.

	VA	Q	MA	NC	FL
Q	**0.13**				
MA	**0.06**	**0.04**			
NC	0.32	0.18	0.12		
FL	0.31	0.24	0.18	0.14	
RK	0.21	0.15	0.12	0.19	0.11

VA, Wachapreague, VA; Q, Qingdao, China; MA, New England; NC, Harker's Island, NC; FL, Crystal River, FL; RK, Rabbit Key, FL. Values in boldface represent samples from this study.

ginia broodstock since the founding of the Chinese line. The number of individuals spawned to produce the sampled population from China is not known but, based on the relative magnitude of the operation, is presumed to be higher than that used in Virginia. It may be that it is simply the scale of the operation that leads to a greater maintenance of diversity. That is, the Chinese may be maintaining multiple lines with low or no diversity, rather than one. Conversely, it may be that instead of fewer mass spawnings, progeny from many, relatively small spawning events are pooled, as recommended by Gaffney et al. (1992), to prevent loss of variation by genetic drift.

Genetic divergence between the two cultured populations is difficult to assess, particularly given the monotypic character of the Virginia samples. Cultured bay scallops from China were not found to share a common gene pool with the putative Virginia source population, and given the lack of any shared mtDNA haplotypes, this finding is not surprising. Corrected mean nucleotide sequence divergences (Table 2) indicate that the bay scallops in the Chinese sample were least divergent from those in the New England sample. It is likely that at the time the scallops were sent to China, the Virginia population also contained a genetic component resembling that found in New England. The introduction of New England bay scallops into the Virginia broodstock is known to have taken place, although it has generally been assumed that the majority of the stock originated from animals that set naturally off North Carolina and Virginia's Eastern Shore. The presence in the Chinese sample of two haplotypes not found in the other sampled populations may also indicate that some rare genotypes, missed in the sampling of the natural populations, were present in the broodstock sent to China but disappeared in the Virginia line. The lack of a sample from the putative Texas source population (from which introductions were made to the Virginia line) makes the possible presence of Texas genotypes in the Chinese sample impossible to evaluate.

It is difficult, in conclusion, to say much about mtDNA variation in either of these cultured lines beyond what can be measured in the current population, because there are periods in the development of both in which the origin of broodstock or the methods of breeding are unclear. This lack of information underscores the importance of good hatchery recordkeeping, and the loss of diversity in both cases highlights the need for a careful breeding regimen that maximizes the number of parental contributors in a broodstock. If one or few spawnings are used to replace the Virginia broodstock for the subsequent generations, then a monotypic lineage will likely persist and problems associated with inbreeding depression may become more apparent. This may also be a problem, more slow to develop but likely more catastrophic to the industry if it does occur, in the Chinese culturing operation.

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LITERATURE CITED

Blake, S. G. & J. E. Graves. 1995. Mitochondrial DNA variation in the bay scallop, *Argopecten irradians* (Lamarck), and the calico scallop, *Argopecten gibbus* (Dall). *J. Shellfish Res.* 14:79–85.

Castagna, M. & W. Duggan. 1971. Rearing the bay scallop, *Aequipecten irradians*. *Proc. Natl. Shellfish. Assoc.* 61:80–85.

Chew, K. K. 1990. Global bivalve introductions. *World Aquacult.* 21:9–22.

Clarke, A. H., Jr. 1965. The scallop superspecies *Aequipecten irradians* (Lamarck). *Malacologia* 2:161–188.

Elder, J. K. & E. M. Southern. 1983. Measurement of DNA length by gel electrophoresis II: comparison of methods for relating mobility to fragment length. *Anal. Biochem.* 128:227.

Gaffney, P. M., C. V. Davis & R. O. Hawes. 1992. Assessment of drift and selection in hatchery populations of oysters (*Crossostrea virginica*). *Aquaculture* 105:1–20.

Gilbert, D. G. 1989. Gel Frag Sizer. dogStar Software, Bloomington, IN.

Ibarra, A. M., P. Cruz & B. A. Romero. 1995. Effects of inbreeding on growth and survival of self-fertilized catarina scallop larvae, *Argopecten circularis*. *Aquaculture* 134:37–47.

Marelli, D. C., W. G. Lyons, W. S. Arnold & M. K. Krause. 1997. Subspecific status of *Argopecten irradians concentricus* (Say, 1822) and of the bay scallops of Florida. *The Nautilus* 110:42–44.

McElroy, D., P. Moran, E. Bermingham & I. Kornfield. 1991. REAP: The Restriction Enzyme Analysis Package. University of Maine, Orono, ME.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York. 512 pp.

Nei, M. & J. C. Miller. 1990. A simple method for estimating average number of nucleotide substitutions within and between populations from restriction data. *Genetics* 125:873–879.

Newkirk, G. F. 1978. A discussion of possible sources of inbreeding in hatchery stock and associated problems. pp. 93–100. *In:* J. W. Avault, Jr. (ed.). Proceedings of the Ninth Annual Meeting of the World Mariculture Society, Louisiana State University, Baton Rouge, LA.

Peterson, C. H. & H. C. Summerson. 1992. Basin-scale coherence of population dynamics of an exploited marine invertebrate, the bay scallop: implications of recruitment limitation. *Mar. Ecol. Prog. Ser.* 90:257–272.

Petuch, E. J. 1987. New Caribbean Molluscan Fauna. The Coastal Education and Research Foundation, Charlottesville, VA. 158 pp.

Roff, D. A. & P. Bentzen. 1989. The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. *Mol. Biol. Evol.* 6:539–545.

Sambrook, J., E. F. Fritsch & T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Shumway, S. E. & M. Castagna. 1994. Scallop fisheries, culture and enhancement in the United States. *Memoirs Queensland Museum* 36:283–298.

Waller, T. R. 1969. The evolution of the *Argopecten gibbus* stock (Mollusc: Bivalvia), with emphasis on the Tertiary and Quaternary species of eastern North America. *J. Paleontol.* 43(suppl. to no. 5):1–125.